# ACTIVATION OF LIVER PYRUVATE KINASE BY FRUCTOSE-1-PHOSPHATE

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## 1. Introduction

Hess, Haeckel and Brand [1] found that fructose-1, 6-diphosphate (FDP)* is an activator of yeast pyruvate kinase (EC 2.7.1.40). A similar effect has been reported for liver pyruvate kinase [2, 3]. Glucose-6-phosphate, fructose-6-phosphate, and 6-phosphogluconate were effective only at concentrations far above those occurring *in vivo* [2]. No activation was found with fructose-1-phosphate and F-6-P under the conditions used in the pyruvate kinase assay [3]. In the course of studies on the metabolism of fructose we have found that F-1-P is an activator of pyruvate kinase at concentrations which occur in rat liver during perfusion with 10 mM fructose.

## 2. Material and methods

Substrates and purified enzymes were obtained from Boehringer Corporation (London) Ltd. The concentration of the F-1-P solutions were determined enzymically [4]; all the hexose monophosphates tested were found to be free of FDP when analysed by the methods of Bücher and Hohorst [5]. Rat liver homogenates, prepared with 4 vols. 1 mM EDTA and stored at –18°C, served as the source of pyruvate kinase. The stock homogenate was thawed at room temperature and diluted 80-fold with distilled water. Pyruvate kinase activities were measured in a reaction mixture which routinely included hexokinase and glucose to remove ATP as this is a competitive inhibitor of liver pyruvate kinase [3].

Table 1
Activations of rat liver pyruvate kinase by hexose phosphates.

| Hexose phosphate added (mM) | | Percentage increases in rate over a control without hexose phosphate |
|---|---|---|
| FDP | 0.005 | 285 |
| | 0.05 | 437 |
| | 0.5 | 485 |
| F-1-P | 0.5 | 41 |
| | 2.5 | 146 |
| | 5.0 | 214 |
| | 10.0 | 368 |
| G-1-P | 0.5 | 26 |
| | 2.5 | 36 |
| | 5.0 | 104 |
| | 10.0 | 195 |
| F-6-P | 0.5 | 29 |
| | 2.5 | 300 |
| | 5.0 | 328 |
| | 10.0 | 346 |
| G-6-P | 0.5 | 15 |
| | 2.5 | 171 |
| | 5.0 | 282 |
| | 10.0 | 342 |

Details of the assay are given in the text. The assay mixture contained 0.25 mg liver and 0.05 mM PEP. ATP was removed by the hexokinase reaction. The above data refer to the period between 10 and 18 min after addition of PEP.

* FDP, fructose-1,6-diphosphate; F-1-P, fructose-1-phosphate; F-6-P, fructose-6-phosphate; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; PEP, phosphoenolpyruvate.

Table 2
Concentrations of hexose phosphates in freeze-clamped rat livers before or after perfusion with 10 mM D-fructose.

| Hexose phosphate | μmoles hexose phosphate/g liver ± S.E.M. | |
|---|---|---|
| | Livers not perfused | Livers perfused for 40 min |
| FDP | 0.017 ± 0.002 (4) | 0.026 ± 0.009 (4) |
| F-1-P | 0.226 (2) | 7.68 ± 0.98 (4) |
| G-1-P | <0.01 (4) | 0.014 ± 0.001 (4) |
| F-6-P | 0.064 ± 0.009 (4) | 0.112 ± 0.008 (4) |
| G-6-P | 0.250 ± 0.032 (4) | 0.378 ± 0.038 (4) |

Livers from rats fed on a normal diet were freeze-clamped in liquid $N_2$ within 5 sec of interruption of circulation. Similar rats had their livers perfused *in situ* with 10 mM D-fructose for 40 min before freeze-clamping [6]. The data above are means ± S.E.M. and the number of animals examined.

The spectrophotometer cuvettes contained: 1.5 ml 0.1 M K phosphate buffer pH 7.4; 0.3 ml 0.1 M $MgCl_2$; 0.07 ml 0.004 M NADH; 0.1 ml 0.04 M ADP; 0.05 ml 0.1 M glucose; 10 μl each of hexokinase and lactic dehydrogenase (Boehringer); 0.1 ml dilute homogenate; and water or hexose phosphate solution to a vol. of 2.9 ml. The mixture was allowed to stand for 2 min to remove any ATP in the added preparation, through the hexokinase reaction, and the pyruvate kinase reaction was started by the addition of 0.1 ml phosphoenolpyruvate solution. In experiments where ATP was added to the cuvettes (in order to make the conditions similar to those in liver in respect to ATP), hexokinase and glucose were omitted. Readings of extinction at 340 nm were taken at 2 min intervals, usually for 18 min at 25°C. In the absence of PEP, removal of NADH by other reactions in these low dilutions of liver was always negligible.

## 3. Results

At higher concentrations (2.5 mM and above) all five phosphates activated pyruvate kinase under the test conditions (table 1), but at concentrations which occur *in vivo* (table 2) only FDP and F-1-P were effective.

The activation of pyruvate kinase by F-1-P was greatest at PEP concentrations occurring in fresh freeze-clamped and perfused rat liver (0.05–0.25 mM). At higher PEP concentrations (0.8 mM) activation by F-1-P no longer occurs (table 3). Weber et al. [3] who found no activation with 2.67 mM F-1-P were using 33.3 mM pEP in their assay mixture. When the concentration of PEP was low and no

Table 3
Effect of FDP and F-1-P on rat liver pyruvate kinase with low and high concentrations of phosphoenolpyruvate.

| Expt. No. | Initial concn. PEP (mM) | Hexokinase and glucose added | Period of measurement after addition of PEP (min) | Pyruvate formed (nmoles/3 ml) | | | % activations by | |
|---|---|---|---|---|---|---|---|---|
| | | | | With no hexose-P added | With 0.05 mM FDP | With 5 mM F-1-P | FDP | F-1-P |
| 1 | 0.10 | none | 2–10 | 18.8 | 63.1 | 48.2 | 236 | 156 |
| | | | 10–18 | 6.8 | 57.9 | 40.5 | 752 | 495 |
| 2 | 0.10 | added | 2–10 | 26.0 | 52.5 | 42.4 | 102 | 63 |
| | | | 10–18 | 13.5 | 48.6 | 35.7 | 260 | 164 |
| 3 | 0.80 | none | 2–10 | 122 | 102 | 86.8 | 0 | 0 |
| | | | 10–18 | 112 | 105 | 89.6 | 0 | 0 |

Details of the assay conditions are given in the text.

hexose phosphate was added the rate of the reaction decreased with time, being less than half in the second 8 min period (table 3). This was partly due to the accumulation of ATP and its inhibition of pyruvate kinase, because the decline was diminished by the addition of hexokinase plus glucose. With F-1-P or FDP the decline in the rate was slight because the hexose phosphate overcomes the inhibition of pyruvate kinase by ATP. Consequently the percentage activation was greatest during the second 8 min period.

Pyruvate kinase assays were also tested (without added hexokinase and glucose) at metabolite concentrations resembling those found [6] in the fed liver perfused for 40 min with fructose (initial concentration 10 mM in the perfusate). This situation is similar to that after intraperitoneal administration of fructose [7]. The concentrations chosen were 0.17 mM PEP, 0.84 mM ATP and 1 mM fructose, with or without the addition of 0.027 mM FDP and 10 mM F-1-P. Effects similar to those shown in table 3 were found after an initial lag period during which activation gradually rose, and rate increases up to 400% were obtained during the 10–18 min period. Under these conditions the effects of FDP and F-1-P were not additive, while at lower concentrations (0.01 mM FDP and 1.0 mM F-1-P) the activations by both were additive.

Pyruvate kinase activity of rat kidney homogenate was less sensitive to F-1-P than the liver enzyme, activation by 5 mM F-1-P was only about 25%. Crystalline rabbit muscle pyruvate kinase (Boehringer) and the enzyme of rat leg muscle homogenate were not activated by F-1-P at all.

## 4. Discussion

The factors which regulate the activity of pyruvate kinase (the concentration of PEP, ADP, ATP, FDP and F-1-P) all vary considerably with the physiological state of the liver [6]. The combined effects of such variations are difficult to compute, but as the conditions under which F-1-P activates pyruvate kinase occur *in vivo* after fructose loading, this activation is likely to play a role in the metabolism of fructose. Fructose can be glycolysed (via pyruvate kinase) more rapidly than glucose [8]. The maximum rate of lactate formation from fructose in the perfused liver of fed rats is up to 4 $\mu$mole/min/g [6] and the activity of pyruvate kinase when assayed under physiological concentrations of PEP (see table 3) is of the same order. It is therefore to be postulated that the activation of pyruvate kinase by F-1-P is essential for maximal rates of fructolysis.

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